A heterothermic spectrum in hummingbirds

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Summary Statement

We find that three hummingbird species are capable of a temperature spectrum from normothermy to torpor, implying that some birds, at least, can precisely control their metabolic state.

Abstract

Many endotherms use torpor, saving energy by a controlled reduction of their body temperature and metabolic rate. Some species (e.g., arctic ground squirrels, hummingbirds) enter deep torpor, dropping their body temperatures by 23-37°C, while others can only enter shallow torpor (e.g., pigeons, 3-10°C reductions). However, deep torpor in mammals can increase predation risk (unless animals are in burrows or caves), inhibit immune function, and result in sleep deprivation, so even for species that can enter deep torpor, facultative shallow torpor might help balance energy savings with these potential costs. Deep torpor occurs in three avian orders, but the trade-offs of deep torpor in birds are unknown. Although the literature hints that some bird species (mousebirds and perhaps hummingbirds) can use both shallow and deep torpor, little empirical evidence of such an avian heterothermy spectrum within species exists. We infrared imaged three hummingbird species that are known to use deep torpor, under natural temperature and light cycles, to test if they were also capable of shallow torpor. All three species used both deep and shallow torpor, often on the same night. Depending on the species, they used shallow torpor for 5-35% of the night. The presence of a heterothermic spectrum in these bird species indicates a capacity for fine-scale physiological and genetic regulation of avian torpid metabolism.

Introduction

Torpor is an energy saving strategy documented in over 200 species of birds and mammals (Boyles et al., 2020; Ruf and Geiser, 2015). Torpor is a fascinating ability—torpid animals save energy by lowering their metabolic rate and body temperature. These animals are often described as having species-specific minimum torpid body temperatures (between -2 and

29.6°C; Barnes, 1989; Hainsworth and Wolf, 1970; McKechnie and Lovegrove, 2002; Richter et al., 2015; Ruf and Geiser, 2015). Depending on their minimum torpid body temperatures, some animals only use a 'shallow' form of torpor (e.g., fasted doves, body temperature 28 – 36°C; Figure 1c), while others use 'deep' torpor, in which body temperature is low (more than 20°C below normothermic body temperatures, e.g., Arctic ground squirrels, hummingbirds, -2 to 18°C; Figure 1b). Both these states are characterized by lower body temperatures than the 1-2°C drop below resting daytime body temperatures that occurs following a circadian rhythm in normothermic sleep ("nocturnal" or "rest-phase" hypometabolism; Geiser, 2021; Walker et al., 1983).

Much of the relatively recent work on the metabolic torpor spectrum has focused on mammals (Boyles et al., 2020; Kräuchi and Deboer, 2011; Reher and Dausmann, 2021; Ruf and Geiser, 2015; van Breukelen and Martin, 2015). Energetic, neurological (EEG), transcriptomic, and ecological evidence exists for a physiological continuum from shallow to deep torpor in mammals, as found in several ground squirrel species, marmots, and kangaroo rats (Berger, 1984; Canale et al., 2012; Florant and Heller, 1977; Glotzbach and Heller, 1976; Heller, 1979; Heller et al., 1978; Walker et al., 1977; Walker et al., 1979). Some bird species are known to use shallow torpor at night or when fasted, while others regularly use deep torpor (Ruf and Geiser, 2015). Though avian shallow torpor and deep torpor have separately received research attention (Beckers and Rattenborg, 2015; Brigham et al., 2000; Powers et al., 2003; Roth et al., 2010; Wolf and Hainsworth, 1972), the differences and potential trade-offs between these states in birds are poorly studied relative to mammals. There are some hints in the literature that such a metabolic spectrum exists in birds under specific conditions (in mousebirds, Schleucher, 2004). We use the word 'spectrum' rather than 'continuum' here because whether this spectrum is continuous or discrete is unknown (some studies argue that it is a continuum between species, Barclay et al.,

2001; while others argue that it is discrete, McKechnie and Lovegrove, 2002). Though birds constitute 65% of extant endotherms, the data on avian heterothermy are sparse compared to mammalian data (Geiser and Ruf, 1995; McKechnie and Mzilikazi, 2011), as are data on this avian heterothermy spectrum. Of the 42 bird species reported to use daily torpor, only hummingbirds (*Trochilidae*), nightjars (*Caprimulgidae*), and one mousebird (*Coliidae*) species have minimum body temperatures colder than 20°C; the rest use a relatively shallow form of torpor (Ruf and Geiser, 2015). Torpor is identified by myriad thresholds across taxa, and by body temperature, metabolism, and heart rate changes (Boyles et al., 2020; Reher and Dausmann, 2021; Ruf and Geiser, 2015). By necessity these thresholds are species- and sometimes individual-specific (Barclay et al., 2001), because they occur in response to changes in ambient temperatures, fat stores, moonlight, and a variety of other environmental and endogenous factors (Eberts et al., 2021; McKechnie and Lovegrove, 2006; Powers et al., 2003; Ruf and Geiser, 2015; Smit et al., 2011). Exploring the range, variability, and flexibility of avian torpor can help elucidate behavioural and physiological mechanisms underlying thermoregulation, energy regulation and torpor use across vertebrates, and move us closer to understanding the evolution of homeothermy vs. heterothermy.

Deep torpor likely reflects a trade-off between its benefits—an average of 60% energy savings relative to basal metabolic rates (Hainsworth et al., 1977; Shankar et al., 2020)—and its potential costs in mammals such as susceptibility to predation, high rewarming costs, immune suppression, and sleep deprivation (Bouma et al., 2010; Boyles et al., 2020; Carr and Lima, 2013; Deboer and Tobler, 2000; Kräuchi and Deboer, 2011; Palchykova et al., 2006; Prendergast et al., 2002). If these trade-offs are similar in birds, it might be beneficial for birds that use the deepest possible form of torpor to sometimes use a shallower form, to allow moderate energy savings

while minimizing some of the potential costs of deep torpor (Boyles et al., 2013). Yet in contrast to mammals, bird species have been observed to use either shallow or deep torpor, but usually not both (Berger, 1984; Brigham et al., 2000; Hainsworth and Wolf, 1978; Hiebert, 1990; Kruger et al., 1982; McKechnie and Lovegrove, 2002; Ruf and Geiser, 2015). One mousebird species has been described to use both shallow and deep torpor when starved over several days, with their depth of torpor deepening as their energy stores were depleted (McKechnie and Lovegrove, 2000; Prinzinger et al., 1992). However, mousebirds are thought to have diverged early in the avian phylogeny and their unusual combination (among birds) of low-quality plant diet with a relatively small body size makes them physiologically distinct in other ways. It is also possible that they display a form of 'proto-torpor' without the standard entry and rewarming patterns of other avian lineages (Bosque et al., 2017; Downs et al., 2000; McKechnie and Lovegrove, 2000). The rarity of shallow torpor in birds that use deep torpor, and vice versa, would imply that shallow and deep torpor are mutually exclusive and relatively inflexible states. The possible existence of an avian torpor continuum has been hinted at in the literature (McKechnie and Lovegrove, 2002; Smit et al., 2011), but evidence supporting or disproving its existence within species is scarce.

Hummingbirds have long been known to use deep torpor to save energy overnight, with minimum body temperatures varying from 3-22°C (Carpenter, 1974; Hainsworth and Wolf, 1970; Hainsworth et al., 1977; Wolf et al., 2020). One past study reported a shallower form of torpor in hummingbirds, but its experimental conditions may have prevented deep torpor: those birds were maintained at warm temperatures, were not free-living, and were frequently disturbed at night (Morrison, 1962). The ability to use both shallow and deep torpor would indicate that they are facultatively using a range of thermal states. This flexibility in body temperatures is almost never described in birds, but such a capacity could contribute to hummingbirds' ability to thrive under

diverse and variable environmental conditions, from deserts to tropical forests and from sea level to the high Andes, despite their small body size and extreme metabolic demands. Previous work suggested that some larger hummingbird species had more variable metabolic rates than smaller hummingbirds (Shankar et al., 2020; Wolf et al., 2020), and our preliminary data from sites in the high Ecuadorian Andes has also suggested that some hummingbird species there might be using a range of shallow and deep torpor (Shankar et al., unpublished observations).

Here we test whether hummingbirds are capable of shallow torpor by recording nighttime surface temperatures in three species sympatric at sites in Arizona (USA) where nighttime temperatures are cold enough to allow deep torpor. We know from previous work that all three species use deep torpor (Powers et al., 2003; Shankar et al., 2020). We hypothesized that these hummingbirds might facultatively use shallow torpor, either alone or in addition to deep torpor, to balance the energy savings and physiological costs of using deep torpor alone. Hummingbirds appear to delay torpor until they have reached some minimum threshold of energy stores (Hainsworth et al., 1977; Hiebert, 1992; Powers et al., 2003). We therefore expected birds to use shallow torpor in one of two ways: either exclusively with normothermy (Figure 1c), or before entering deep torpor, as a strategy to delay the onset and potential costs of deep torpor (Figure 1d). Given that hummingbirds seem to reach a minimum fat threshold before entering deep torpor (Eberts et al., 2021; Powers et al., 2003), we expected that once a bird entered deep torpor, it would stay in deep torpor for the remainder of that night, and then rewarm to normothermy before flying off, rather than using shallow torpor after deep torpor. We use thermal imaging to study hummingbird torpor under near-natural conditions. This study design allows us to assess torpor use under natural light and temperature cues, as well as near-natural energy stores. Such

physiological control in torpor would in turn imply that a broad and perhaps continuous avian heterothermic spectrum exists, much like in mammals.

Methods

Study sites and species

We studied males of three hummingbird species at the Southwestern Research Station (SWRS) in the Chiracahua mountains of Arizona (Lat: 31.9, Long: -109.2): the blue-throated mountain-gem (*Lampornis clemenciae;* 8.4g, n = 14), Rivoli's hummingbird (*Eugenes fulgens*; 7.6g, n =12) and the black-chinned hummingbird (*Archilocus alexandri*; 2.9g, n = 7). Two blue-throated mountain-gem individuals had some bill corrugation and were likely late-stage juveniles. Within this hummingbird community, both the black-chinned and Rivoli's hummingbirds are subordinate to blue-throated mountain-gems (i.e., with less exclusive access to floral resources) (Powers et al., 2003; Sandlin, 2000). We collected data between June 10 – 19, 2017 and May 20 – June 7, 2018. All protocols associated with hummingbird care and experimentation were approved by the Stony Brook University Institutional Animal Care and Use Committee (IRBNet number: 282617-6). Field protocols were approved by US Fish and Wildlife in Arizona (USFWS MB75714A-0).

Thermal imaging—nighttime surface temperatures

We captured hummingbirds using modified Hall traps at hummingbird feeders (Russell and Russell, 2001) within 1.5 hours before sunset, to allow them to store energy naturally through the day, but also acclimate to our experimental setup. The majority of birds were already banded (this

is a long-term bird monitoring site), but un-banded birds were marked with a unique set of small dots of non-toxic paint on the forehead (2017: 4 birds unbanded, 8 banded; 2018: 6 birds unbanded, 16 banded). We recorded capture mass, allowed the birds to feed *ad libitum*, and weighed them again for mass after feeding. They were then placed outdoors (individually) in five-sided acrylic chambers (either 18x17x22 cm or 46x23x46 cm), exposed to natural light and temperatures. All nighttime recordings were between 1900h-0559h. The front face of the chamber was covered by a clear plastic sheet to prevent the bird escaping. This sheet caused the thermal reading of the bird's surface temperatures to be up to 2°C cooler than readings without the sheet, so once the bird was observed to settle down, the plastic sheet was removed. We placed a wire grill at the base of the chamber to encourage birds to perch with their sagittal plane facing the camera, usually ensuring that recordings included a direct view of the bird's eye. Recordings without this view were excluded from analyses.

Bird eye surface temperatures seem to closely reflect internal physiological state (e.g. body condition), from recent work in blue tits (Jerem et al., 2018). Hummingbirds have low feather density around the eye, so skin eye temperature patterns should closely reflect the patterns of core body temperature, minimizing the confounding effects of feather insulation, unlike in larger animals for which skin and core temperatures might vary because of reduced peripheral blood flow in torpor (Arnold et al., 2011; Barclay et al., 1996; Brigham et al., 2000; Dausmann, 2005; Powers et al., 2015). Powers et al. (2017) used thermal imaging to measure heat dissipation areas in hovering hummingbirds during the day in three species. They found that across all three species, eye surface temperature remained relatively constant across a range of ambient temperatures, with an intercept of 32–33°C (Figure 2 in Powers et al., 2017). Although this is lower than core body temperature, it is consistent with what is observed in individuals from these

same species that are clearly normothermic. This supports our measurements of 32°C being a common resting normothermic body surface temperature. A recent study of hummingbird body temperature in torpor across six species showed that many individuals maintained normothermy at body (cloacal) temperatures between 35.5-40°C (Wolf et al., 2020). Additionally, there seems to be high concordance between eye surface temperatures and metabolic rates as measured in ruby-throated hummingbirds (Erich Eberts, University of Toronto Scarborough, personal communication).

We used a FLIR SC6701 infrared video camera (640 × 480 pixel resolution, accurate to 1°C at measured temperatures, FLIR Systems, Inc., Wilsonville, OR, USA) to record surface temperatures of hummingbirds. We assumed emissivity was 0.95 across all surfaces of the hummingbird (Cossins and Bowler, 1987; Powers et al., 2015). We monitored birds continuously through the night, and sampled surface temperatures by recording 10 seconds of 30Hz video approximately every 10 minutes, using ResearchIR (FLIR Systems, Inc., Wilsonville, OR, USA). From one frame per recording, a region including the bird and a slight buffer to include ambient temperatures was marked as a region of interest and exported to csv files for analysis in R (v.3.5.1; R Core Team, 2018). From each exported region of interest, we extracted maximum surface temperature (in Celsius) and mean surface temperature of the bird, as well as minimum temperature of the background (our proxy for ambient temperature; details below). We verified that maximum surface temperatures corresponded with maximum surface temperatures of the region surrounding the eye (hereafter eye surface temperature though these were never the temperatures of the actual surface of the eye, which is usually colder than the skin/feathers surrounding the eye), and validated outliers in temperature measurements to ensure that they were reliable measurements. We also exported entire single-frame images from selected

recordings and used ImageJ (NIH) to construct 3D images to assess how surface temperatures changed over the entire surface of the bird.

Ambient temperature

We used minimum temperatures from thermal image regions of interest as an estimate of ambient temperatures, verifying that these closely matched independent ambient temperature measurements from iButtons (DS1921, Maxim Integrated, San Jose, CA, USA) or thermocouples (Cu-Cn type-T, recorded on a TC-1000; Sable Systems, Las Vegas, CA, USA). The FLIR camera was factory calibrated and verified by imaging a surface of a known temperature. Thermocouples and iButtons were calibrated by using a Percival (i.e., a temperature-controlled cabinet, model I-35LV, Percival Scientific, Inc., Perry, IA, USA) at controlled temperature steps, and checked against a thermometer traceable to the National Institute of Standards and Technology.

Thermal categories

We assigned bird surface temperature measurements at each time point to one of four categories: normothermy, shallow torpor, transition to and from deep torpor, or deep torpor. We defined these categories using individually-assigned thresholds for each bird based on the values and patterns of eye surface temperature and ambient temperature. We used eye surface temperatures of the bird once it had settled, but its eyes were still open, to define resting normothermic temperatures per individual. Once the eyes were closed, we considered the bird asleep (Mascetti, Gian Gastone, 2016). If eye surface temperatures dropped more than 2°C below these resting temperatures (Walker et al., 1983), we visually classified the birds into one of the

other three categories based on 1. rate of temperature change (stable, slow change, rapid change), and 2. magnitude of decrease of eye surface temperature below normothermic temperature, and above ambient temperature.

Birds were considered in shallow torpor if they dropped more than 2°C but less than 20°C below their resting temperature (but were still above ambient temperature), and maintained that temperature for more than 10 minutes (stable temperatures). These thresholds are certainly somewhat arbitrary, but they clearly overlap a range of temperatures that have been previously defined by others to be torpor (Geiser, 2021). Past studies have advocated for individual-specific thresholds to define normothermy and torpor (Barclay et al., 2001). An equivalent of shallow torpor has previously also been referred to as nocturnal hypothermia (e.g., McKechnie and Lovegrove, 2002), but more recent work suggests using the term 'hypothermia' only for a pathological condition that an animal cannot rewarm from by itself, and using 'torpor' for controlled body temperature reduction with independent rewarming (Geiser et al., 2014). Normothermy has been defined as a state when body temperatures are within ±2°C of resting body temperature (Hetem et al., 2016). Thus, normothermy is, by definition, not associated with the flexibility in body temperatures that these birds are clearly capable of; and at these times that we defined as shallow torpor, they are not at their minimum torpid body temperatures. From past usages of the term shallow torpor (Berger, 1984; Geiser, 2021; Jonasson and Willis, 2012), shallow torpor is the most appropriate term for what we observed. Measurements were assigned to the transition category if they dropped or increased rapidly between normothermy and deep torpor, or between shallow and deep torpor (i.e., transitions were defined by rapid, large temperature changes; average rate of change ± s.e. of 0.45 ± 0.06°C/min, up to 3°C/min; see Figure S2 for details). Birds were considered to be in deep torpor if eye surface temperature was close to

ambient temperature, or if it was maintained below 20°C without dropping any lower (stable, low temperatures), for an extended period (highest reported hummingbird torpid body temperature is 22°C; Bech et al., 1997; Hainsworth and Wolf, 1970). This is by no means steady-state torpor, but does cover a range of temperatures that is not normothermy. Categories varied slightly across individuals because we assigned category thresholds per bird based on its surface temperature patterns relative to resting and ambient temperatures, and based on the rate of temperature change.

Surface temperatures models

Normothermy, shallow torpor and deep torpor could be distinguished by the relationship between eye surface (response) and ambient (predictor) temperature. While a normothermic homeotherm can maintain a relatively stable body or surface temperature over a large range of ambient temperatures, in deep torpor the body and surface temperatures become a positive function of ambient temperature. Therefore, we would expect deep torpor to have a steep slope and a very low intercept. If shallow torpor exists, then the slopes for normothermy and shallow torpor should be similar and low, while their intercepts should vary (shallow torpor lower than normothermy). Additionally, we expected species to use these torpor categories differently, and expected mass to negatively influence torpor use (birds with greater energy stores should use torpor less, Powers et al., 2003).

To estimate regression equations of surface temperature as a function of ambient temperature for each of the four thermal categories (normothermy, shallow torpor, transition, deep torpor) we used linear mixed effects models (Gelman and Hill, 2006) using the 'nlme'

package in R (Bates et al., 2015). A mixed effects model is appropriate because the response (surface) can be modelled as a function of various data types; in this case both continuous fixed effects (ambient temperature, thermal categories, mass, species, and year), as well as random effects (categories nested within individuals) and an autocorrelation term were incorporated. To first test the effect of ambient temperature on surface temperature, we ran a simple linear model of surface temperature (T_S) as a function of ambient temperature (T_a). This model only explained 15% of the variation in surface temperatures, and we therefore ran an 'lme' linear mixed effects model of surface temperature as a function of ambient temperature. We included mass as a continuous fixed covariate; thermal category (normothermy, shallow torpor, etc.), species, and year as discrete fixed covariates, and categories within individuals as a random covariate. We included interaction terms between category and both ambient temperature and species. We also included an autocorrelation term ('CorAR1'; see Supplement 1 for model details):

$$T_s \sim T_a * Category + \ Species * Category + Capture\ mass + Year + \left(1 \middle| \frac{Individual}{Category}\right) + CorAR1$$

Frequency of thermal category use

To estimate the proportion of time that each species spent in each of the four categories, we calculated the proportion of the night spent in each thermal category for every individual. We then modelled the percentage of the night spent in each category per species. We ran thermal category and species as interacting terms because we expected them to have interactive effects. The model was:

$$Category\ frequency \sim Category * Species$$

We ran a generalized linear model, fitting a negative binomial distribution to the data (Venables and Ripley, 2002). We first ran Poisson and quasipoisson models, but both were overdispersed (see Supplement 1 and Table S1). We therefore fit a negative binomial which was much better fit than the others (Table S2). We used the 'glm.nb' function in the MASS package in R to run this model (R Core Team, 2018; Venables and Dichmont, 2004; Venables and Ripley, 2002).

Results

Ambient temperatures

Ambient temperatures usually declined steadily over the course of the night (e.g., in Figure 2). In 2017, temperatures were an average ± s.d. of 13 ± 4.6°C (range 3 to 23°C), and in 2018 they were 11 ± 5.6°C (range -1 to 24.6°C). Most nights ranged between 5 – 20°C (mean 12°C), except for one particularly cold night with ambient temperatures between -1 and 14°C (May 20, 2018), and one especially warm night (June 5, 2018) during which ambient temperatures ranged between 15 – 25°C.

Nighttime surface temperatures

The surface temperatures of normothermic birds and birds in shallow torpor peaked near the eye and decreased from the eye towards the tail (Figures 2 and 3). Birds in deep torpor were evenly cold. Nighttime eye surface temperature varied overall between 5.9-38°C (See 'Individual plots' supplement). Active birds at the beginning of the night had normothermic temperatures ranging between 31-38°C. This wide range included birds that were flying around in the chamber

before settling and birds at rest. When they settled down, normothermic temperatures usually stabilized (when the bird was resting with eyes open) at 31°C, so we usually considered minimum normothermic resting surface temperatures to be around 31°C. In some cases, birds stabilized at 29°C, at both the start and end of the night, with minimal fluctuation; in these cases we set the resting normothermic threshold to be 29°C. Maximum eye surface temperatures ranged from 29-38°C in normothermy, to 19.5–29°C in shallow torpor, and 5.9–24.1°C in deep torpor.

Surface temperature model results

The full model for surface temperature (where the slopes and intercepts vary by category and species) allowed us to identify and quantify the various thermal categories, including shallow torpor (Figure 3, Table S3 and Table S4). Mass did not seem to have a large effect surface temperature given the other factors, but year did.

In this model of the factors affecting surface temperature, the normothermic and shallow torpor categories had similar, very low, slopes (0.11), while the normothermy intercept was about 4°C higher than the shallow torpor intercept. A 4°C drop from normothermy has previously been categorized as being a form of torpor (Ruf and Geiser, 2015). In contrast with these thermoregulating states, hummingbirds largely thermoconform in deep torpor (down to the ambient temperatures we measured them at, which were all above their minimum body temperatures in deep torpor). In deep torpor, their surface temperatures closely tied to ambient temperature (slope of 0.85) and a low intercept about 20°C lower than the normothermy intercept). The transition category is a non-equilibrial physiological state, with an intermediate intercept 17°C lower than normothermy.

Frequency of thermal category use

Shallow torpor was used by all species, but at varying frequencies (Figure 4, Figure S1). Of the 34 individuals we studied, all 34 were normothermic for part of the night; 25 used shallow torpor for part of the night; 8 individuals used shallow but not deep torpor; 20 transitioned between deep torpor and normothermy, and 20 used deep torpor (Figure 4a).

All seven black-chinned hummingbirds (BCHU) used deep torpor, for an average of 49% of the night, while only three of these individuals used shallow torpor, for an average of 5% per night. BCHU spent 34% of the night on average in normothermy, and 12% in transition. The 14 blue-throated mountain-gems (BLUH) remained largely normothermic (67% of the total nighttime), and used shallow torpor an average of 25% of the night, with four individuals remaining normothermic all night, 10 individuals using shallow torpor for at least some time and only four using deep torpor (4% of the night), with 3% of the night spent on transitioning on average. The 12 Rivoli's hummingbirds (RIHU) were the most variable in their use of the various metabolic states, with 10 individuals using shallow torpor (33% of total nighttime), six individuals using all four categories, four using normothermy and shallow torpor, one individual remaining normothermic all night, and one individual using all categories except shallow torpor. The RIHU individuals spent an average of 43% of the night in normothermy, 8% in transition, and 17% in deep torpor.

The model of thermal category frequency across species showed that there were overall clear differences in the thermal categories across all species. There were also species-specific differences in the use of normothermy, shallow torpor, deep torpor and the transition categories

(Figure 4b and Table S3). The model resulted in estimates that were more evenly distributed than the raw data (the two panels on Figure 4b), but the differences between species were still clear.

The two blue-throated mountain-gems that we studied on an especially warm night maintained high surface temperatures all night (33-35°C). Another two blue-throated mountain-gem individuals that could have been late-stage juveniles appeared to behave similarly to adults: one used all four categories, and the other used only normothermy and shallow torpor. Contrary to our expectations, several Rivoli's hummingbirds and a few blue-throated mountain-gems used shallow torpor for 1-2 hours after coming out of deep torpor, while the black-chinned hummingbirds never used shallow torpor after deep torpor.

Discussion

We describe and quantify the novel use of shallow torpor in birds that are known to use deep torpor. Similar to mammals, and in contrast with previous studies that either describe birds as using shallow torpor or deep torpor, hummingbirds appear capable of using both. Hummingbirds in shallow torpor appear to thermoregulate to maintain surface temperatures below normothermy. In contrast, hummingbirds in deep torpor largely thermoconform to ambient temperatures. The intermediate shallow state could serve to balance nighttime energy savings with the potential ecological and physiological costs of deep torpor. Reflecting what previous studies have found (Powers et al., 2003), birds with larger energy stores seem to have greater flexibility in avoiding deep torpor. The two larger species in our study used normothermy and shallow torpor for a greater proportion of the night than the smaller species. Our minimally invasive study design allowed us to thermally image hummingbirds under near-natural

temperature cycles, without disturbing or touching the birds through the night, and allowed us to discover a new level of flexibility in hummingbirds' management of their nighttime energetic needs.

All three species used all thermal categories, but unequally. Rivoli's hummingbirds used shallow torpor the most, followed by blue-throated mountain-gems. The small black-chinned hummingbirds used shallow torpor the least. It therefore appears that black-chinned hummingbirds, the smallest of the three study species, might have the least flexibility in managing their nighttime energy budget, while blue-throated mountain-gems, the large territorial species, have the most flexibility. Individuals of the two larger species appeared to have more flexibility in regulating their nighttime body temperature, commonly using shallow torpor or a combination of shallow and deep torpor. The more limited use of deep torpor in these two species is consistent with previous findings that these species tend to avoid deep torpor (Powers et al., 2003; Shankar et al., 2020).

Our results support the argument that there must be either physiological or ecological costs of deep torpor (Boyles et al., 2020; Ruf and Geiser, 2015), because hummingbirds that are clearly capable of using deep torpor sometimes use shallow torpor or avoid torpor altogether. Using shallow torpor rather than deep torpor could be especially beneficial in three scenarios. First, deep torpor in mammals (especially hibernation) is usually considered helpful in avoiding predation because torpid animals are less conspicuous to predators; but these animals are usually hidden in hibernacula or dens (Ruf and Geiser, 2015; Turbill et al., 2011). Torpid birds in trees, in contrast, might be more conspicuous, making shallow torpor more efficient than deep torpor in allowing them to respond to potential predators (Carr and Lima, 2013). Hummingbirds in shallow

torpor could afford quicker rewarming times (< 5 minutes), and quicker responses to predators or other external stimuli, relative to deep torpor for which rewarming to normothermy takes an average of 20-30 minutes (Bucher and Chappell, 1992; Shankar et al., 2020). Some mammals in torpor and even birds at least in shallow torpor (e.g., Common Poorwills; Austin and Bradley, 1969) are capable of locomotive activity (Geiser, 2021). Second, at least in mammals, the physiological costs of torpor include rewarming costs, immune suppression (Bouma et al., 2010; Prendergast et al., 2002), increased oxidative stress (Buzadžić et al., 1997), and potential sleep deprivation (Kräuchi and Deboer, 2011; Palchykova et al., 2002). There are hints that daily heterothermic mammals (Djungarian hamsters, *Phodopus sungorus*; 26g) enter a euthermic state after torpor to recover from sleep deprivation (Deboer and Tobler, 2000; Palchykova et al., 2002). Shallow torpor would allow higher levels of metabolic function than deep torpor, perhaps facilitating some of the restorative functions of sleep, immunity, and lowered oxidative stress. Though avian sleep has been studied to some extent (Beckers and Rattenborg, 2015), little is known about the physiological basis for torpor vs. sleep in birds. Third, for nesting birds that need to keep their nest warm, shallow torpor could help balance the birds' need to maintain energy balance with the need to supply heat to their eggs or chicks. Nesting hummingbirds have been found to generally avoid torpor (with exceptions when energy stores seemed to be low); but the use of shallow torpor by nesting birds has not been evaluated (Calder, 1971; Howell and Dawson, 1954; Smith et al., 1974). If deep torpor had no ecological or physiological consequences, hummingbirds would likely maximize torpor use, or remain in deep torpor for the duration of the night after entering torpor. Instead, some individuals used shallow torpor not just before a deep torpor bout as we predicted, but after emerging from a deep torpor bout, indicating that they may

be trying to save energy but also balance these energy savings with the potential costs of deep torpor.

Here we identified four thermal categories in hummingbirds: two thermoregulatory categories—normothermy and shallow torpor; a thermoconforming category—deep torpor; and the transition between deep torpor and the other categories. Though we have assigned them discrete names, it is thus far unclear whether the underlying physiological differences between these states in birds are continuous or discrete. In normothermy and shallow torpor the animal actively thermoregulates to maintain a constant body temperature across a range of ambient temperatures. Based on the similar surface temperature slopes of normothermy and shallow torpor (Figure 3), and the rapid transitions between normothermy and shallow torpor that we often observed, these two states seem metabolically continuous in hummingbirds (as they seem to be in doves; Walker et al., 1983). Shallow torpor, as defined here, could potentially be a metabolically inhibited form of normothermic sleep, but it is unclear whether the shallow torpor and deep torpor we report are on a similar metabolic spectrum, and we were unable to definitively distinguish sleep using only body temperature measurements. Multiple lines of evidence, especially in ground squirrels and pocket mice, from electroencephalograms (EEGs), measurements of brain temperature, and metabolic rates indicate that mammals slow their metabolism continuously down from sleep into torpor, and regulate their body temperatures variably above minimum body temperature (Berger, 1984; Boyles and McKechnie, 2010; Boyles et al., 2013; Ruf and Geiser, 2015; Walker et al., 1979). Thus, though sleep and torpor appear to be on a continuous spectrum in mammals, it remains to be seen whether they are on a continuum in birds.

Given that hummingbirds can regulate between shallow and deep torpor, the biological relevance of minimum body temperature measurements must be assessed. Recent work with high-elevation Andean hummingbirds found that minimum torpid body temperature showed a phylogenetic signal, indicating that minimum torpid body temperature, at least at very cold sites, is evolutionarily conserved (Wolf et al., 2020). Shallow torpor can occur either because a bird's minimum possible torpid body temperature is relatively high (i.e., it does not have the capacity for deeper torpor), or when a bird regulates at a high, sub-normothermic, body temperature despite its minimum torpid body temperature being much lower (e.g., 15°C, indicating that it is capable of deep torpor, Figure 1d). These two shallow torpor scenarios are indistinguishable (as in Figure 1c) unless the species' "true" minimum body temperature is known. If a bird regulates at a body temperature above its minimum, even though ambient temperatures were lower, these measurements might appear to be minimum body temperature measurements although they are not. In Rivoli's hummingbirds, for instance, we found that eye surface temperatures went as low as 5.9°C. However, Rivoli's individuals in the laboratory were previously reported to regulate their minimum body temperature at 12°C despite ambient temperatures going lower (Wolf and Hainsworth, 1972). Such a large disparity in birds in deep torpor is unlikely to be due to differences between core and skin temperatures, and could either indicate intra-specific differences in minimum body temperature, or that the birds in the previous study were using a shallower form of torpor. This disparity could also be caused by birds reducing their blood circulation around the eye during torpor, but such regional variation seems unlikely given the small size of hummingbirds and the even distribution of low surface temperatures we observed in torpid birds. Minimum body temperature may therefore be lower than has been reported in some species. Currently minimum body temperature across all hummingbirds is thought to vary from

3–22°C (Bech et al., 1997; Carpenter, 1974; Wolf et al., 2020). But if some of the hummingbirds measured were using shallow rather than their deepest possible torpor, the range of hummingbirds' true minimum body temperatures would be narrower or lower. Additionally, torpor studies in hummingbirds are often conducted in laboratory conditions, which could alter torpor responses (McKechnie and Lovegrove, 2002; Ruf and Geiser, 2015).

We propose three reasons for why this form of shallow torpor in birds has so rarely been detected (Prinzinger et al., 1992). First, small drops in oxygen consumption or body temperature might have been overlooked. Second, most studies of bird torpor are either done under controlled laboratory conditions, or involve handling the birds many times at night to record body temperature. Birds in captivity are often overweight and have to be starved to enter torpor (Schleucher and Prinzinger, 2006). Laboratory torpor studies conducted at controlled temperature steps might have pre-empted the use of shallow torpor, because shallow torpor is presumably a fine-scale response to energetic state and environmental conditions, and controlled temperature steps or repeated handling might not elicit the same physiological responses as natural decreases in nighttime temperature would (Geiser et al., 2000). Third, birds in laboratory settings are known to show altered torpor use relative to free birds: free-living animals often use torpor more frequently, and drop to a lower body temperature in torpor than laboratory animals (McKechnie and Lovegrove, 2002; McNab, 1989; reviewed in Ruf and Geiser, 2015). Taken together, under relatively predictable natural temperature patterns, hummingbirds might be able to use intermediate torpor states more often, while in the laboratory, low temperatures, the factor most often tested, might cause the bird to either stay awake or drop into deep torpor if energetically necessary.

At the whole-animal level, the next step in understanding avian torpor would be to combine respirometry, thermal measurements and measurements of breathing or heart rates while keeping in mind the possible existence of shallow torpor. These measures have been found to sometimes be uncoupled in torpor (O'Mara et al., 2018; Toien et al., 2011), and therefore studying whether they vary together in shallow torpor would be the first step in identifying the physiological differences between sleep, shallow torpor and deep torpor. A promising future avenue for research would be to investigate which metabolic and genetic pathways shut down at different temperatures in hummingbird torpor. It remains to be seen if other hummingbird and bird species that use deep torpor are also capable of shallower torpor, or if such control over their torpid metabolism is unique to these two hummingbird clades. Our data indicate that these hummingbird species in a temperate environment with cold ambient temperatures often use shallow torpor; it is therefore possible that tropical species at sites with high ambient temperatures might be doing the same.

Author contributions

AS, DRP, CHG and INHC were involved in study conception, design and obtaining funding. AS, INHC, ST and DRP collected data; AS analysed the data and wrote the manuscript, CHG and DRP provided major comments and revisions.

Acknowledgements

Data and Code accessibility

The R scripts used to run the analyses are available on Github: https://github.com/nushiamme/TorporShallowDeep. The data associated with this manuscript are available on Dryad (https://doi.org/10.5061/dryad.cc2fqz65h or a "for review" link: https://datadryad.org/stash/share/Mrf3RsqjzJIMak6Btc8q-1FljMu63XIEr9gmwGv03v0).

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Figures
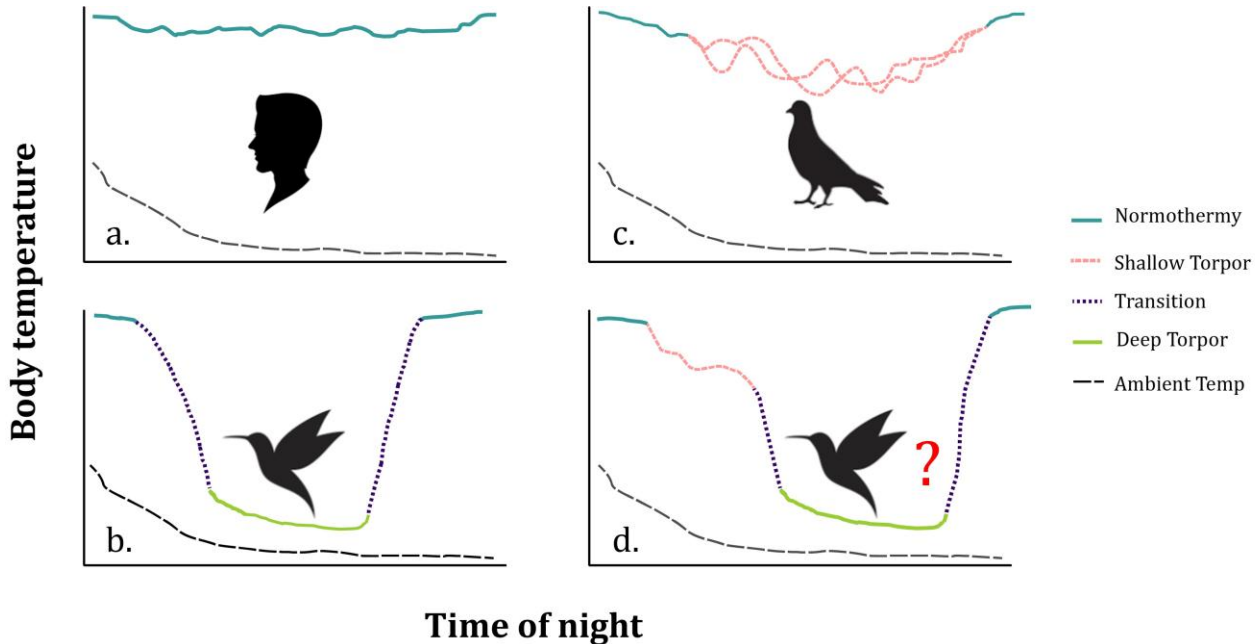
Figure 1: A schematic depiction of body temperature (coloured lines) relative to ambient temperature (black dashed line) at night, in sleep, shallow torpor, and deep torpor. a. A normothermic individual, with minimal circadian reductions in nighttime body temperature (e.g., humans). b. An individual starts the night normothermic, then transitions into deep torpor, where body temperature drops with ambient temperature, minimizing the difference between minimum body temperature and ambient temperature (e.g., hummingbirds). c. An individual starts the night normothermic, then uses 'shallow' torpor, potentially because the species has a very high minimum body temperature of only 4-5°C below normothermic levels (e.g., some pigeon species). This use of shallow torpor can show a variety of patterns, either stabilizing or oscillating up and down (alternative pink dashed lines). d. An individual uses a combination of normothermy, shallow, and deep torpor, at times regulating its body temperature above its minimum torpid body temperature – here we investigate the presence of such a heterothermy spectrum in hummingbirds.
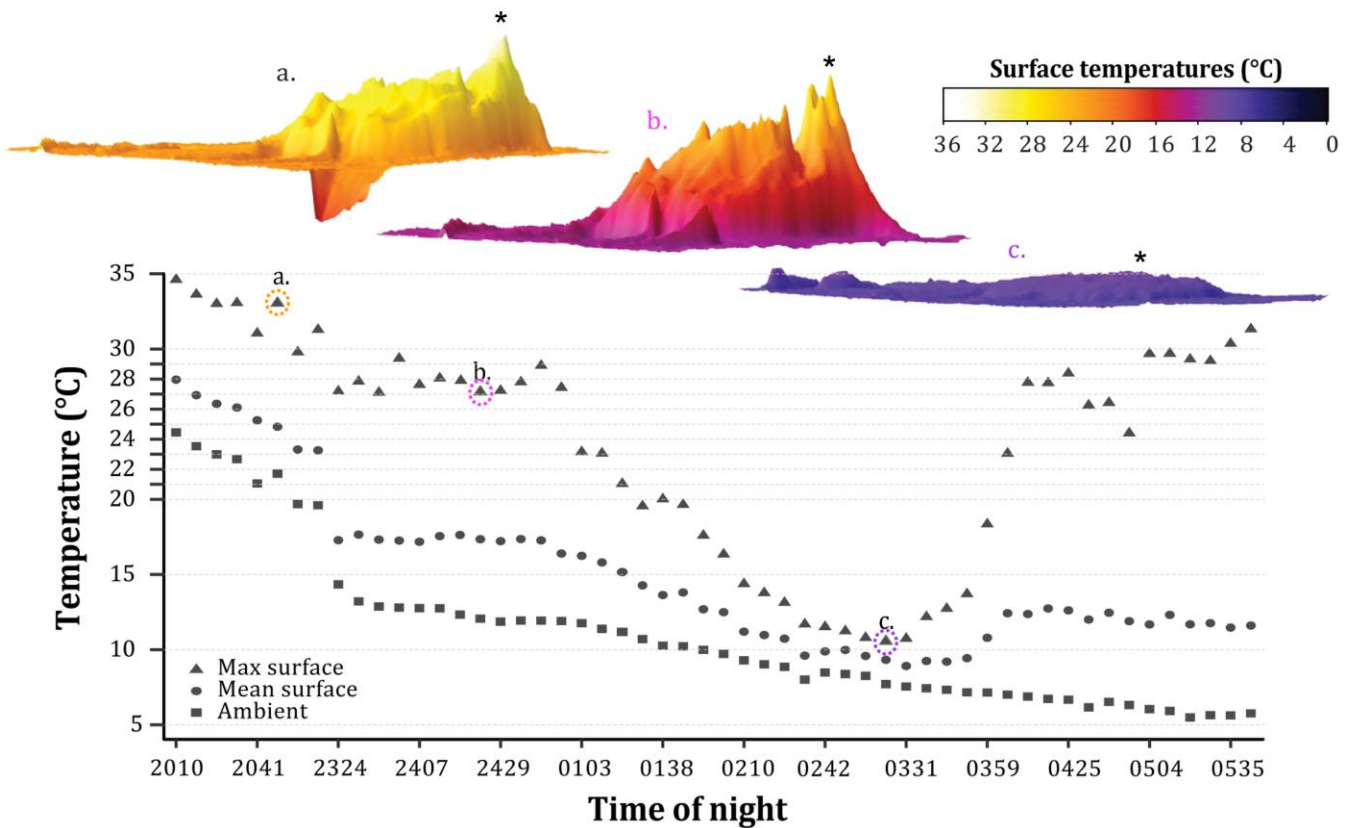
Figure 2: A Rivoli's hummingbird using all four metabolic states (normothermy, shallow torpor, transition and deep torpor). Top: 3D plots of the surface temperature of the bird in normothermy, shallow torpor, and deep torpor, aligned with the tail-beak axis along the x-axis. Asterisks indicate the location of the eye. See Supplementary Video SV1 for perspective on the 3D plots. a. Normothermic: surface temperatures peak at 35°C near the eye; mean surface temperature is 25°C. b. Shallow: surface temperatures peak around the eye at 27°C, followed by a drop in temperature and steady, much lower, surface temperatures over the rest of the body (17°C), and then a steady drop towards the tail. c. Torpor: The entire surface of the bird is cold, peaking around the eye at 11°C.
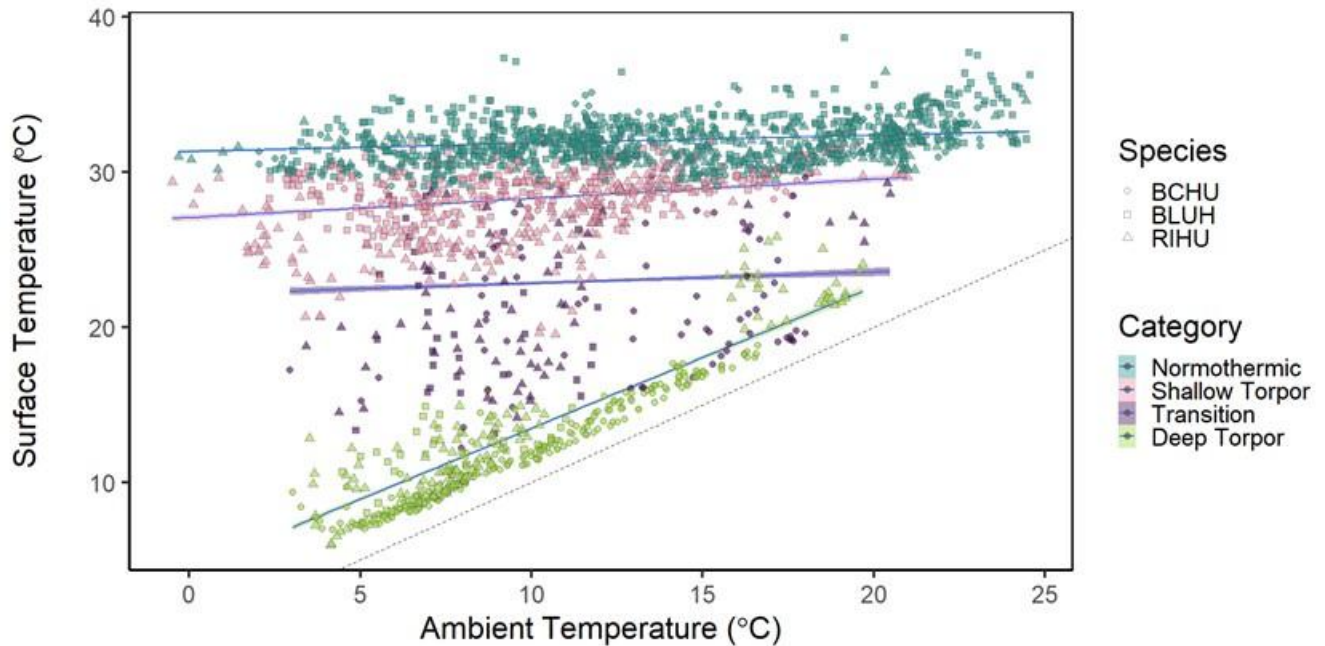
Figure 3: Predicted model fit from the linear mixed effects model of maximum surface temperatures (eye temperatures) as a function of ambient temperatures, coloured by category. As we predicted, deep torpor had a steep slope and low intercept, while shallow torpor and normothermy had similar low slopes and high intercepts (see Tables S3 and S4 for regression coefficients). The dashed line is an identity line to shown where surface temperatures would equal ambient temperatures. BCHU = black-chinned hummingbird; BLUH = blue-throated mountain-gem; RIHU = Rivoli's hummingbird.
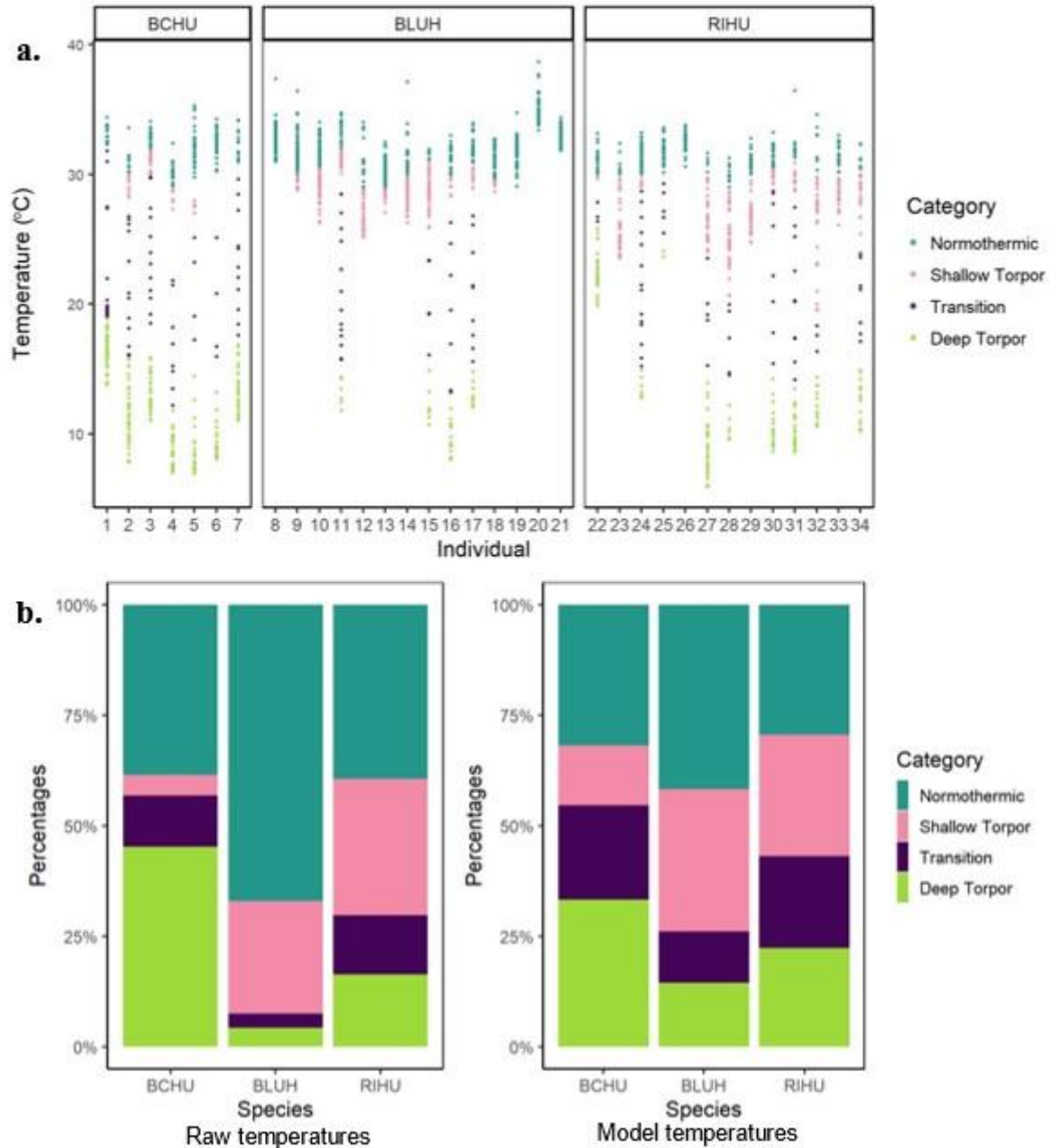
Figure 4: **a**. Distribution of values recorded per category (colours), per individual. Each column is a single night from one individual. BCHU = black-chinned hummingbird; BLUH = blue-throated mountain-gem; RIHU = Rivoli's hummingbird. **b.** The relative percentages of time a species spent over all nights studied in each of the four categories: normothermic, shallow torpor, transition, torpor. BCHU = black-chinned hummingbird; BLUH = blue-throated mountain-gem; RIHU =

Rivoli's hummingbird. Left: Percentages calculated using raw data. Right: Model estimates from the glm model (Category frequency ~ Category*Species - 1), of the relative time per category per species, presented as percentages.
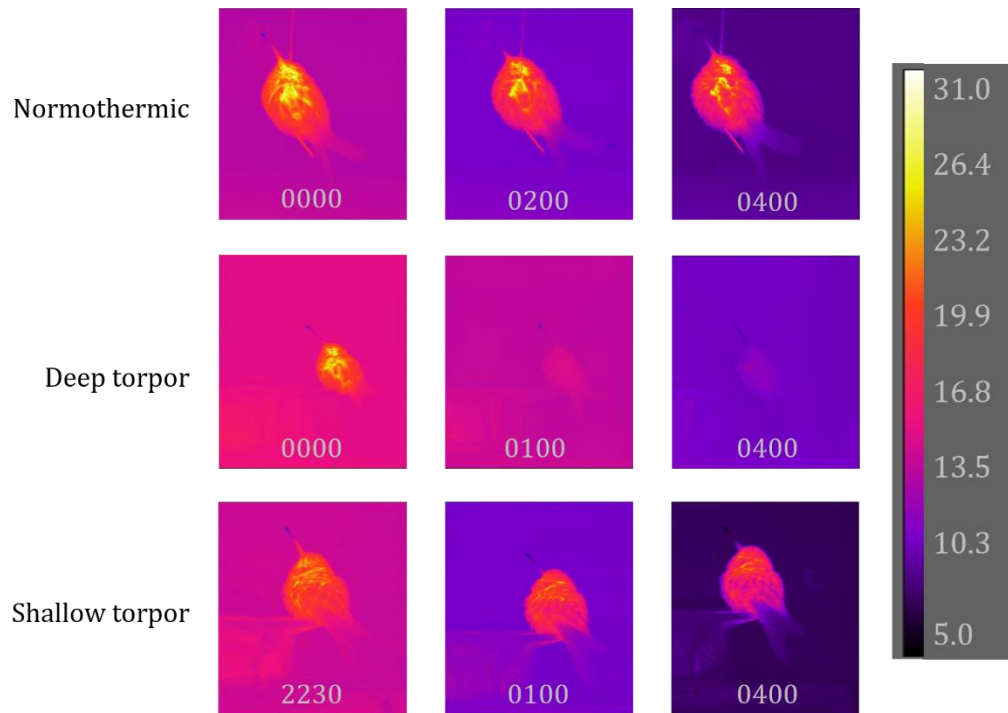
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Fig. S1. Thermal images of individuals in all the categories except 'Transition'. Top: Blue-throated mountain-gem that remained normothermic all night (Surface temperature peaking at 31-32 °C). Middle: black-chinned hummingbird that was normothermic at midnight and then entered deep torpor and was torpid the rest of the night (15 ℃). Bottom: Rivoli's hummingbird that was normothermic at 2230, but then entered shallow torpor for much of the night.

Supplementary Methods:

Models of frequency of use of a thermal category: We first ran a Poisson distribution because the data were a form of frequency or count data (frequency of categories per species across all nights), but the Poisson caused overdispersion, with the residual variance being much higher than the degrees of freedom (see Table S1). We then fit a quasipoisson model, but the model was still overdispersed, with a dispersion parameter of 12.54 (a dispersion parameter great than one means the model is overdispersed). We therefore fit a negative binomial, which resulted in a dispersion parameter of 0.69 (Table S2). This model was therefore a much better fit than either of the others.

Models of surface temperature: To first test the effect of ambient temperature on surface temperature, we ran a simple linear model of surface temperature (T_S) as a function of ambient temperature (T_a), without incorporating the other variables. This model only explained 15% of the variation in surface temperatures, and we therefore ran a linear mixed effects model of surface temperature as a function of ambient temperature. We included mass as a continuous fixed covariate, and thermal category (normothermy, shallow torpor, etc.), year (because ambient temperatures varied across the two years), and species as discrete covariates. We modelled categories within individuals as a random covariate. We included a correlation structure ('corAR1') to account for temporal autocorrelation, with categories nested within individuals; and interaction effects between category and species and between ambient temperature and category (Table S3, Table S4).

$$T_s \sim T_a * Category + \ Species * Category + Capture\ mass + Year + \left(1 \left| \frac{Individual}{Category} \right. \right)$$

$$+ CorAR1$$

We tested for normality of the residuals, homogeneity of variances, and linearity to confirm that the model was a good fit.

Table S1. Model results of the Poisson model of differences between the species' use of the four thermal categories (normothermic, shallow, transition, deep torpor). The model was $Category\ frequency \sim Category * Species - 1$ such that intercepts and slopes were allowed to vary by category and by species. The estimates were very similar to those of the negative binomial model, but this model was overdispersed, and was therefore not used.

Category	Species	Estimate	SE	Lower CL	Upper CL
Normothermic	BCHU	3.65	0.06	3.53	3.77
Shallow Torpor	BCHU	1.55	0.17	1.21	1.89
Transition	BCHU	2.45	0.11	2.23	2.67
Deep Torpor	BCHU	3.81	0.06	3.70	3.92
Normothermic	BLUH	4.20	0.03	4.14	4.27
Shallow Torpor	BLUH	3.24	0.05	3.13	3.34
Transition	BLUH	1.17	0.15	0.88	1.46
Deep Torpor	BLUH	1.46	0.13	1.20	1.71
Normothermic	RIHU	3.68	0.04	3.59	3.76
Shallow Torpor	RIHU	3.43	0.05	3.33	3.53
Transition	RIHU	2.59	0.08	2.45	2.74
Deep Torpor	RIHU	2.80	0.07	2.66	2.93
Null deviance: 19670 on 136 degrees of freedom Dispersion parameter for Poisson family taken to be 1					
Residual deviance: 2252 on 124 degrees of freedom AIC: 2770					

Table S2. Model results of the negative binomial model testing differences between the species' use of the four thermal categories (normothermic, shallow, transition, deep torpor). The model was $Category\ proportion \sim Category * Species - 1$ such that intercepts and slopes were allowed to vary by category and by species. These frequency estimates are only meaningful in relation to one another, and can be compared by converting them into proportions within a species (as depicted in Figure 5b on the right). All the estimates' confidence intervals do not overlap with zero.

| Species	Estimate	SE	Lower CL	Upper CL
BCHU Normothermic	3.65	0.48	2.70	4.59
BCHU Shallow Torpor	1.55	0.51	0.55	2.55
BCHU Transition	2.45	0.49	1.49	3.41
BCHU Deep Torpor	3.81	0.48	2.87	4.75
BLUH Normothermic	4.20	0.34	3.54	4.87
BLUH Shallow Torpor	3.24	0.34	2.56	3.91
BLUH Transition	1.17	0.37	0.44	1.89
BLUH Deep Torpor	1.46	0.36	0.75	2.16
RIHU Normothermic	3.68	0.35	2.98	4.37
RIHU Shallow Torpor	3.43	0.35	2.73	4.12
RIHU Transition	2.59	0.36	1.89	3.30
RIHU Deep Torpor	2.80	0.36	2.10	3.50
Null deviance: 2888 on 136 degrees of freedom Dispersion parameter for Negative Binomial (0.6627) family taken to be 1				
Residual deviance: 161.5 on 124 degrees of freedom AIC: 1066				
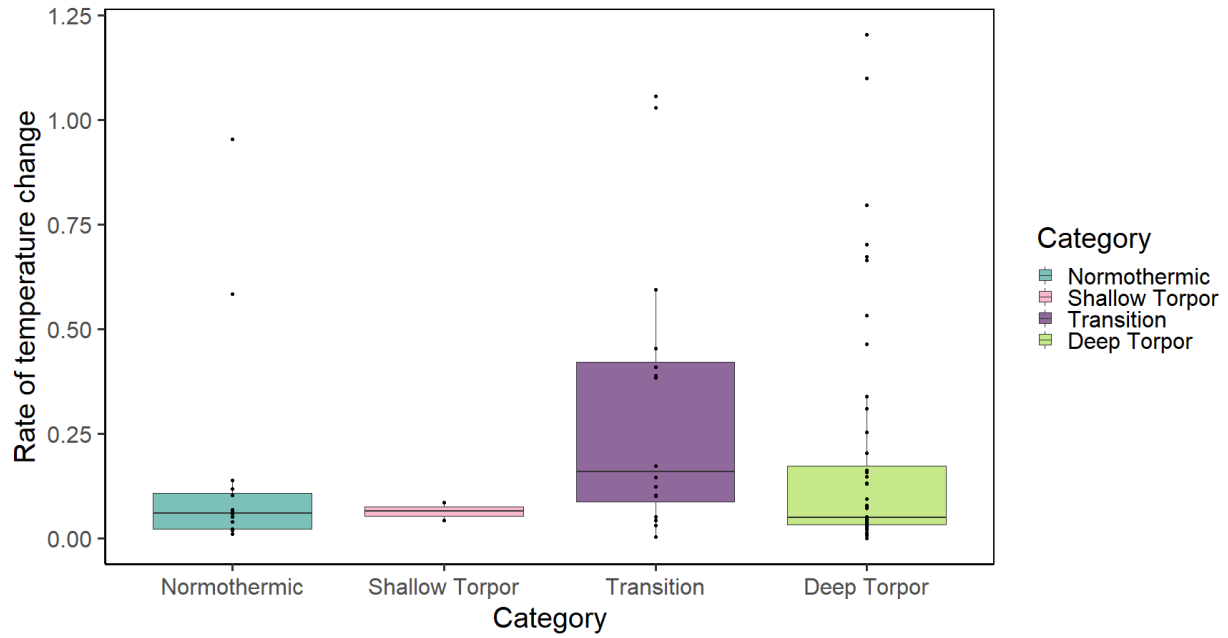
Fig. S2. Absolute rates of temperature change per category in °C/minute.

Table S3. Model outputs of the surface temperature model. These are mean estimates of each thermal category per species, with standard error, degrees of freedom, and lower and upper confidence limits.

Species	Category	Mean	SE	df	Lower CL	Upper CL
BCHU	Normothermic	33.05	1.83	29	29.31	36.78
BLUH	Normothermic	31.80	0.67	29	30.43	33.16
RIHU	Normothermic	31.03	0.51	29	29.98	32.08
BCHU	Shallow Torpor	30.41	1.92	29	26.48	34.34
BLUH	Shallow Torpor	28.97	0.78	29	27.38	30.57
RIHU	Shallow Torpor	27.64	0.50	29	26.62	28.66
BCHU	Transition	21.54	1.85	29	17.75	25.33
BLUH	Transition	22.22	1.02	29	20.13	24.30
RIHU	Transition	22.47	0.63	29	21.18	23.77
BCHU	Deep Torpor	15.08	1.82	29	11.35	18.80
BLUH	Deep Torpor	15.41	1.03	29	13.29	17.53
RIHU	Deep Torpor	15.40	0.57	29	14.22	16.57

Table S4. Summary of the full surface temperature model results for each term in the model. T_a = ambient temperature.

Variable	Value	SE	DF	t-val	p-val
(Intercept)	46.53	5.99	2062	7.77	1.2e-14
T_a	0.06	0.01	2062	4.10	4.2e-05
Category Shallow Torpor	-3.06	0.90	56	-3.41	1.2e-03
Category Transition	-17.31	0.90	56	-19.19	2.7e-26
Category Deep Torpor	-26.43	0.81	56	-32.66	0.0e+00
Species BLUH	-1.25	2.42	29	-0.52	6.0e-01
Species RIHU	-2.02	2.21	29	-0.91	3.6e-01
Capture mass	0.30	0.43	29	0.69	4.9e-01
Year	-0.93	0.34	29	-2.70	1.1e-02
T_a : Category Shallow Torpor	0.04	0.03	2062	1.27	2.0e-01
T_a : Category Transition	0.49	0.05	2062	9.91	1.1e-22
T_a : Category Deep Torpor	0.71	0.06	2062	12.27	1.9e-33
Category Shallow Torpor : Species BLUH	-0.18	0.94	56	-0.19	8.4e-01
Category Transition : Species BLUH	1.92	1.04	56	1.85	6.9e-02
Category Deep Torpor : Species BLUH	1.58	0.97	56	1.64	1.0e-01
Category Shallow Torpor : Species RIHU	-0.75	0.95	56	-0.79	4.3e-01
Category Transition : Species RIHU	2.95	0.89	56	3.30	1.7e-03
Category Deep Torpor : Species RIHU	2.34	0.78	56	2.99	4.1e-03
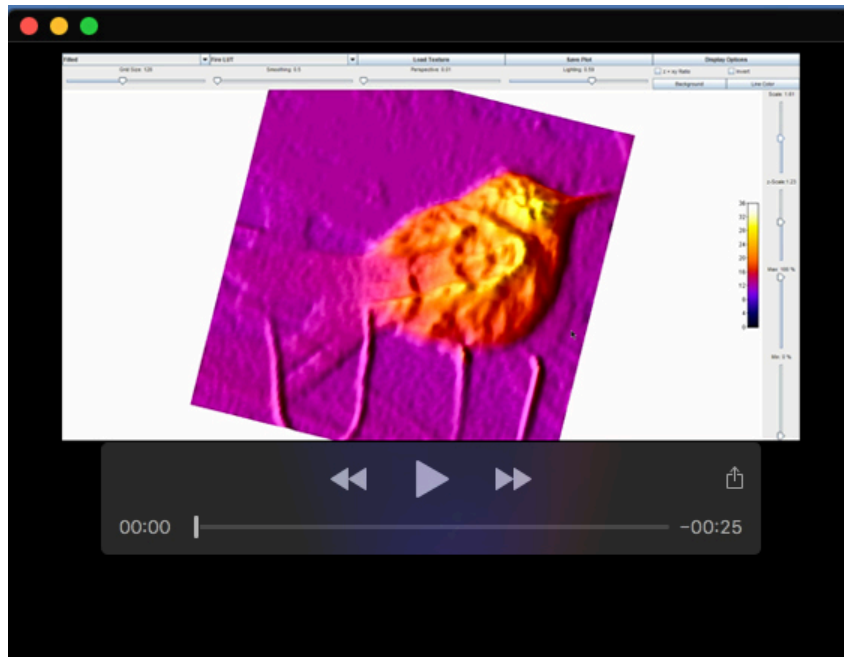
Movie 1. A rotation of a 3D thermal image of a hummingbird to a 2D view of distributions of temperatures over the surface of a hummingbird (accompanies Figure 2).